NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 433

TOXICOLOGY AND CARCINOGENESIS STUDIES OF TRICRESYL PHOSPHATE

(CAS NO. 1330-78-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE AND FEED STUDIES)

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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NATIONAL TOXICOLOGY PROGRAM
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CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and report findings

K.M. Abdo, Ph.D.
C.J. Alden, Ph.D.
G.A. Boorman, D.V.M., Ph.D.
D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
S.L. Eustis, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
R.D. Irwin, Ph.D.
G.N. Rao, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
B.A. Schwetz, D.V.M., Ph.D.
C.C. Shackelford, D.V.M., M.S., Ph.D.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Battelle Columbus Laboratories

Conducted 16-day and 13-week studies, evaluated pathology findings

A.C. Peters, D.V.M., Principal Investigator
M.J. Ryan, D.V.M., Ph.D.
P.C. Stromberg, D.V.M., Ph.D.

Conducted 2-year studies, evaluated pathology findings

P.J. Kurtz, Ph.D., Principal Investigator
M.J. Ryan, D.V.M., Ph.D.
A.W. Singer, D.V.M.

Experimental Pathology Laboratories, Inc.

Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
K. Yoshitomi, D.V.M., Ph.D.
E. Gaillard, D.V.M.

Dynamac Corporation

Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group

Evaluated slides, prepared pathology report on rats (9 October 1991)

P.K. Hildebrandt, D.V.M., Chair
PATHCO, Inc.
W.W. Carlton, D.V.M., Ph.D.
Purdue University
J.R. Hailey, D.V.M.
National Toxicology Program
M.M. McDonald, D.V.M., Ph.D.
National Toxicology Program
R.C. Sills, D.V.M., Ph.D.
National Toxicology Program
K. Yoshitomi, D.V.M., Ph.D.
Experimental Pathology Laboratories

Evaluated slides, prepared pathology report on mice (18 September 1991)

J.R. Leininger, D.V.M., Ph.D., Chair
Pathology Associates, Inc.
E. Gaillard, D.V.M.
Experimental Pathology Laboratories
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
M.P. Jokinen, D.V.M.
National Toxicology Program
J. Kanno, M.D., Ph.D.
Tokyo Medical and Dental University
M.M. McDonald, D.V.M., Ph.D.
National Toxicology Program
R. Miller, D.V.M.
Chemical Industry Institute of Toxicology

Biotechnical Services, Inc.

Prepared Technical Report

D.D. Lambright, Ph.D., Principal Investigator
J.R. Beverly, B.A.
P. Chaffin, B.S.E.
G. Gordon, M.A.
E.S. Rathman, M.S.

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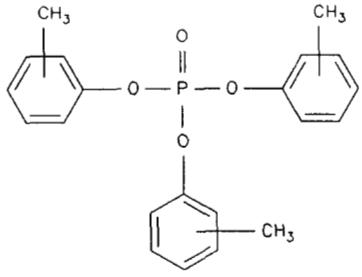
ABSTRACT



TRICRESYL PHOSPHATE

CAS No. 1330-78-5

Chemical Formula: $C_{21}H_{21}O_4P$ Molecular Weight: 368.36

Tricresyl phosphate is an organophosphate plasticizer widely used in vinyl plastics and as a fire retardant additive for hydraulic fluids. Toxicology and carcinogenesis studies were conducted by administering a mixed isomer preparation of 79% tricresyl phosphate esters (consisting of 21% tri-*m*-cresyl phosphate, 4% tri-*p*-cresyl phosphate, less than 1% tri-*o*-cresyl phosphate, and other unidentified tricresyl phosphate esters) by gavage to groups of F344/N rats and $B6C3F_1$ mice for 16 days and 13 weeks, and in feed to groups of F344/N rats and $B6C3F_1$ mice for 13 weeks and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and cultured Chinese hamster ovary cells.

16-Day Gavage Study in Rats

Groups of 10 male and 10 female rats received tricresyl phosphate in corn oil by gavage at doses of 0, 360, 730, 1,450, 2,900, or 5,800 mg/kg body weight, 5 days per week, for a total of 13 or 14 doses in a 16-day period. One female receiving 1,450 mg/kg and five males and eight females receiving 2,900 mg/kg died before the end of the study. Final mean body weights of male and female rats that received 1,450, 2,900, or 5,800 mg/kg were significantly lower than those of the controls. Necrosis of the mandibular lymph node, spleen, and thymus occurred primarily in rats receiving 2,900 and 5,800 mg/kg. Diffuse aspermatogenesis occurred in the testes of male rats that received 2,900 and 5,800 mg/kg. Changes in neurobehavioral parameters in groups that received 1,450, 2,900, or 5,800 mg/kg were confounded by mortality and reduced body weights and were not attributed to a direct neurotoxic response.

16-Day Gavage Study in Mice

Groups of 10 male and 10 female mice received tricresyl phosphate in corn oil by gavage at doses of 0, 360, 730, 1,450, 2,900, or 5,800 mg/kg body weight, 5 days per week, for a total of 13 or 14 doses in a 16-day period. Five males and all females that received 1,450 mg/kg, all mice that received 2,900 mg/kg, and four males and one female that received 5,800 mg/kg died before the end of the study. Final mean body weights of male mice that received 1,450 and 5,800 mg/kg were significantly lower than that of the controls. Final mean body weights of female mice that received 360, 730, or 5,800 mg/kg were significantly greater than that of the controls. Necrosis of the mandibular lymph node, thymus, and spleen occurred primarily in mice receiving 2,900 and 5,800 mg/kg. Hindlimb grip strengths of male mice that received 360 and 1,450 mg/kg and male and female mice that received 730 and 5,800 mg/kg were significantly lower than those of the controls at the end of the study.

13-Week Gavage Study in Rats

Groups of 10 male and 10 female rats received tricresyl phosphate in corn oil by gavage at doses of 0, 50, 100, 200, 400, or 800 mg/kg body weight. All rats survived to the end of the study. Final mean body weights of male rats receiving 200, 400, and 800 mg/kg were significantly lower than that of the controls. Cytoplasmic vacuolization of the adrenal cortex occurred in all dosed groups and the severity increased with dose. Ovarian interstitial cell hypertrophy occurred in all dosed groups of females. Atrophy of the seminiferous tubules occurred in male rats that received 400 and 800 mg/kg. There were no biologically significant changes in neurobehavioral parameters in rats.

13-Week Gavage Study in Mice

Groups of 10 male and 10 female mice received tricresyl phosphate in corn oil by gavage at doses of 0, 50, 100, 200, 400, or 800 mg/kg body weight. All mice survived to the end of the study. Final mean body weights of male mice receiving 200 mg/kg and of male and female mice receiving 400 and 800 mg/kg were significantly lower than those of the controls. Cytoplasmic vacuolization of the adrenal cortex occurred in all dosed groups of mice and the severity increased with dose. Ovarian interstitial cell hypertrophy was present in all dosed groups of female mice. Multifocal degeneration of the spinal cord occurred in males and females that received 100, 200, 400, and 800 mg/kg, and multifocal degeneration of the sciatic nerve occurred in males that received 200, 400, and 800 mg/kg and females that received 100, 200, 400, and 800 mg/kg. Hindlimb grip strengths of male mice that received 200, 400, or 800 mg/kg were significantly lower than that of the controls at the end of the study.

13-Week Feed Study in Rats

Groups of 10 male and 10 female rats were fed diets containing 0, 900, 1,700, 3,300, 6,600, or 13,000 ppm of tricresyl phosphate. All rats survived to the end of the study. Final mean body weights of males and females exposed to 6,600 and 13,000 ppm and females exposed to 3,300 ppm were significantly lower than those of controls. Feed consumption by male and female rats exposed to 13,000 ppm was lower than that by controls during the first week of the study. Dietary levels of 900, 1,700, 3,300, 6,600 or 13,000 ppm tricresyl phosphate were estimated to deliver daily doses of 55, 120, 220, 430, or 750 mg/kg body weight (males) and 65, 120, 230, 430, or 770 mg/kg (females). There were no biologically significant changes in neurobehavioral parameters in rats.

Cytoplasmic vacuolization of the adrenal cortex occurred in all exposed groups of rats. Hyperplasia of ovarian interstitial cells and inflammation of the ovarian interstitium occurred in all exposed groups of females. Renal papillary edema and renal papillary necrosis occurred in 13,000 ppm males and females and in 6,600 ppm females. Basophilic hypertrophy of the pituitary gland pars distalis and atrophy of the seminiferous tubules occurred in 6,600 and 13,000 ppm males.

Dose selection for the 2-year study in rats was based on lower mean body weights; toxic responses observed in the kidney, pituitary gland, and testis of males and the kidney of females exposed to 6,600 and 13,000 ppm; the presence of cytoplasmic vacuolization of the adrenal cortex in exposed males and females; and the occurrence of ovarian interstitial cell hyperplasia in females exposed to 900 and 1,700 ppm.

13-Week Feed Study in Mice

Groups of 10 male and 10 female mice were fed diets containing 0, 250, 500, 1,000, 2,100, or 4,200 ppm of tricresyl phosphate. All mice survived to the end of the study. Mean body weights of 4,200 ppm males and of females exposed to 2,100 and 4,200 ppm were lower than those of controls throughout the study. Feed consumption by females exposed to 1,000, 2,100, or 4,200 ppm was lower than that by controls during week 12. Dietary levels of 250, 500, 1,000, 2,100, or 4,200 ppm tricresyl phosphate were estimated to deliver average daily doses of 45, 110, 180, 380, or 900 mg/kg body weight (males) and 65, 130, 230, 530, or 1,050 mg/kg (females). Interpretation of grip strength changes observed in groups receiving 2,100 or 4,200 ppm were confounded by the reduced body weights of these groups.

Cytoplasmic vacuolization of the adrenal cortex occurred in all exposed groups of male and female mice with the exception of 250 ppm males. Papillary hyperplasia of the gallbladder mucosa occurred in male mice exposed to 500 ppm or more and in female mice exposed to 1,000 ppm or more. Axonal

degeneration occurred in males and females exposed to 2,100 and 4,200 ppm and females exposed to 1,000 ppm. Renal tubule regeneration occurred in all 4,200 ppm male mice.

Dose selection for the 2-year study in mice was based on the presence of axonal degeneration at concentrations of 1,000 ppm or more and cytoplasmic vacuolization of the adrenal cortex at concentrations of 500 ppm or more in males and in all exposed groups of females.

2-Year Feed Study in Rats

Groups of 95 male and 95 female rats were fed diets containing 0, 75, 150, or 300 ppm of tricresyl phosphate. An additional group of 95 male and 95 female rats were fed diets containing 600 ppm of tricresyl phosphate for 22 weeks and then received only control feed. After 3, 9, and 15 months of chemical exposure, up to 15 males and 15 females per group were evaluated for forelimb and hindlimb grip strength, then necropsied and evaluated for histopathologic lesions.

Survival, Mean Body Weights, and Feed Consumption

Survival of exposed rats was similar to that of controls. The final mean body weights of all exposed groups of males and females were similar to those of the controls. Feed consumption by exposed groups of male and female rats was similar to that by the controls. Dietary levels of 75, 150, or 300 ppm tricresyl phosphate were estimated to deliver average daily doses of 3, 6, or 13 mg/kg body weight (males) and 4, 7, or 15 mg/kg (females).

Pathology Findings

There were no chemical-related increased incidences of neoplasms in rats. Cytoplasmic vacuolization of the adrenal cortex occurred in 600 ppm males and 150, 300, and 600 ppm females at the 3-month interim evaluation. At 9 and 15 months, cytoplasmic vacuolization occurred only in female rats, primarily in the 300 ppm group. Cytoplasmic vacuolization of the adrenal cortex and ovarian interstitial cell hyperplasia occurred in female rats exposed to 300 ppm throughout the 2-year study and the incidence and severity were significantly increased at the end of the study.

2-Year Feed Study in Mice

Groups of 95 male and 95 female mice were fed diets containing 0, 60, 125, or 250 ppm of tricresyl phosphate. After 3, 9, and 15 months of chemical exposure, up to 15 males and 15 females per group were evaluated for forelimb and hindlimb grip strength, then necropsied and evaluated for histopathologic lesions.

Survival, Mean Body Weights, and Feed Consumption

Survival of exposed groups of male and female mice was similar to that of the controls. The final mean body weights of males and females receiving tricresyl phosphate were similar to those of controls. Feed consumption by exposed groups of male and female mice was similar to that by the controls. Dietary levels of 60, 125, or 250 ppm tricresyl phosphate were estimated to deliver average daily doses of 7, 13, or 27 mg/kg body weight (males) and 8, 18, or 37 mg/kg (females).

Pathology Findings

There were no chemical-related increased incidences of neoplasms in mice. Ceroid pigmentation of the adrenal cortex occurred in all groups of mice throughout most of the 2-year study, with the exception of 60 and 125 ppm females at the 3-month interim evaluation; however, the severity was markedly increased in female mice receiving 250 ppm. Incidences of clear cell foci, fatty change, and ceroid pigmentation of the liver were significantly increased in male mice that received 125 or 250 ppm.

Genetic Toxicology

Tricresyl phosphate was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, nor did it induce chromosomal aberrations or sister chromatid exchanges in cultured Chinese hamster ovary cells. These *in vitro* assays were all conducted with and without exogenous metabolic activation (S9).

Conclusions

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of tricresyl phosphate in male or female F344/N rats that received 75, 150, or 300 ppm. There was *no*

evidence of carcinogenic activity of tricresyl phosphate in male or female B6C3F$_1$ mice that received 60, 125, or 250 ppm.

Nonneoplastic lesions associated with exposure to tricresyl phosphate included cytoplasmic vacuolization of the adrenal cortex and ovarian interstitial cell hyperplasia in female rats, increased incidences of clear cell focus, fatty change, and ceroid pigmentation of the liver in male mice, and increased severity of ceroid pigmentation of the adrenal cortex in female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Tricresyl Phosphate

Variable	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 75, 150, or 300 ppm in feed (Approximately 3, 6, or 13 mg/kg)	0, 75, 150, or 300 ppm in feed (Approximately 4, 7, or 15 mg/kg)	0, 60, 125, or 250 ppm in feed (Approximately 7, 13, or 27 mg/kg)	0, 60, 125, or 250 ppm in feed (Approximately 8, 18, or 37 mg/kg)
Body weights	Exposed groups similar to controls	Exposed groups similar to controls	Exposed groups similar to controls	Exposed groups similar to controls
2-Year survival rates	32/51, 30/50, 35/50, 28/50	34/51, 38/53, 30/50, 26/49	43/51, 43/49, 44/49, 42/50	41/50, 38/50, 42/48, 45/51
Nonneoplastic effects	None	Adrenal cortex: cytoplasmic vacuolization (14/51, 12/53, 16/50, 36/50); Ovary: interstitial hyperplasia (0/51, 0/53, 0/50, 15/50)	Liver: ceroid pigmentation (0/52, 0/49, 30/49, 28/50); clear cell focus (5/52, 8/49, 17/49, 12/50); fatty change (6/52, 10/49, 23/49, 22/50)	Adrenal cortex: ceroid pigmentation (severity grades - 1.2, 1.6, 2.5, 3.9)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Salmonella typhimurium gene mutation:	Negative with and without S9 in strains TA98, TA100, TA1535, and TA1537			
Sister chromatid exchanges				
Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Chromosomal aberrations				
Chinese hamster ovary cells *in vitro*:	Negative with and without S9			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such lesions to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on tricresyl phosphate on June 22, 1993, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Curtis D. Klaassen, Ph.D., Chair
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Paul T. Bailey, Ph.D., Principal Reviewer
Environmental and Health Sciences Laboratory
Mobil Oil Corporation
Princeton, NJ

Louis S. Beliczky, M.S., M.P.H.*
Department of Industrial Hygiene
United Rubber Workers International Union
Akron, OH

Arnold L. Brown, M.D.
University of Wisconsin Medical School
Madison, WI

Kowetha A. Davidson, Ph.D., Principal Reviewer
Health and Safety Research Division
Oak Ridge National Laboratory
Oak Ridge, TN

Harold Davis, D.V.M., Ph.D.
Medical Research Division
American Cyanamid
Pearl River, NY

Daniel S. Longnecker, M.D.*
Department of Pathology
Dartmouth Medical School
Lebanon, NH

Louise Ryan, Ph.D.
Division of Biostatistics
Howard School of Public Health and
Dana-Farber Cancer Institute
Boston, MA

Ellen K. Silbergeld, Ph.D.*
University of Maryland Medical School
Baltimore, MD

Robert E. Taylor, M.D., Ph.D.
Department of Pharmacology
Howard University College of Medicine
Washington, DC

Matthew J. van Zwieten, D.V.M., Ph.D.,
Principal Reviewer
Department of Safety Assessment
Merck Research Laboratories
West Point, PA

Jerrold M. Ward, D.V.M., Ph.D.
National Cancer Institute
Frederick, MD

Lauren Zeise, Ph.D.
Reproductive and Cancer Hazard Assessment Section
California Environmental Protection Agency
Berkeley, CA

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 22, 1993, the draft Technical Report on the toxicology and carcinogenesis studies of tricresyl phosphate received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of tricresyl phosphate by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related nonneoplastic lesions in rats and mice. The proposed conclusions were *no evidence of carcinogenic activity* of tricresyl phosphate in male or female rats or mice.

Dr. van Zwieten, a principal reviewer, agreed with the proposed conclusions. He thought the description of the rationale for maximum tolerated dose was extremely well done.

Dr. Davidson, the second principal reviewer, agreed with the proposed conclusions. She asked for an explanation why there was high mortality in male and female rats that received 2,900 mg/kg in the 16-day gavage study, while at double that dose there was no mortality. Similar results were observed in mice. Dr. Irwin said the higher dose was pure tricresyl phosphate, which is a liquid, while the lower dose was the chemical diluted with an equal amount of corn oil. He speculated that the corn oil may have enhanced the absorption. Because dosed feed was used in the 2-year studies, this observation was not pursued further.

Dr. Bailey, the third principal reviewer, also agreed with the proposed conclusions. He said that mention needed to be made in the introductory toxicity section that tricresyl phosphate esters with only one *ortho*-cresyl substituent are much more potent neurotoxicants than the tri-*ortho*-cresyl ester. He provided a reference.

Dr. Ryan inquired as to why extensive neurotoxicity testing was reported in an appendix but there was little discussion of the results. Dr. Irwin replied that neurotoxicity was considered to be a possible complicating factor that might interfere with evaluation of carcinogenic potential. Tests such as measurement of grip strength in response to acoustic and thermal stimuli were intended to determine whether there was neurotoxicity present. In public comments, Dr. Mary Barth, Mobil Oil Corporation, reported that there are several unpublished studies that indicate tricresyl phosphate is somewhat more toxic with corn oil as a vehicle than with mineral oil as a vehicle.

Dr. van Zwieten moved that the Technical Report on tricresyl phosphate be accepted with the revision discussed and with the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity*. Dr. Bailey seconded the motion, which was accepted unanimously with nine votes.